Vitamin B-Complex Initiates Growth and Development of Human Embryonic Brain Cells *In Vitro*

K. E. Danielyan, R. A. Abramyan*, A. A. Galoyan, and G. A. Kevorkian

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 5, pp. 513-517, May, 2011
Original article submitted September 6, 2010

We studied a combined effect of subcomponents of vitamin B complex on the growth, development, and death of human embryonic brain-derived cells (E90) cultured using a modified method of Matson. Cell death was detected by trypan blue staining. According to our results, vitamin B-complex in low-doses promote the development, maturation, and enlargement of human embryonic brain cells, on the one hand, and increases the percent of cell death, which attests to accelerated maturation and metabolism, on the other.

Key Words: *vitamin B-complex; human embryo; brain; cell culture*

Biologically active vitamin B-complex containing a certain dose ratio of vitamins B is used in medical practice as a dietary supplement since 1930 [6]; complex treatment with these vitamins is much more effective than their individual application.

Many laboratories studied the effect of individual components of vitamin B complex on the growth and development of the nervous tissue or cell cultures. It was demonstrated that thiamine deficiency can serve as the basis for modeling of chronic oxidative metabolism disorders leading to neurodegeneration [9]. Moreover, thiamine deficiency can cause death of neurons, rather than astrocytes, microglia, or endothelial cells lining the inner wall of blood vessels in the brain [9]. In rats, thiamine deficiency during pregnancy induces death of brain cells. Oxythiamine treatment after thiamine deficiency promoted survival of hippocampal neurons [4]. Antagonists of oxythiamine inhibit this effect. The impact of pyridoxine and its derivative pyridoxal phosphate on survival of nerve cells isolated from the brain was also studied. Pyridoxine and pyridoxal phosphate increased survival of neural cultures with high cell density (10^5 cells/cm^2), while neither pyridoxine, nor pyridoxal phosphate produced similar effect on low-density cell culture (5×10^3 cells/cm^2). Neurotrophic effect of pyridoxal phosphate was suppressed with picrotoxin and ifenprodil. Aminohydroxyacetic acid, an inhibitor of pyridoxal phosphate-dependent enzymes, initiated death of nerve cells by itself. It was assumed that vitamin B6 contributes to neuronal survival by coenzyme stimulation of enzymes responsible for the neurotransmitter synthesis [3]. It was shown that nicotinamide, a NAD precursor, is capable of preventing apoptosis caused by neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Moreover, nicotinamide eliminates free radicals that can be accumulated due to xanthine oxidase activity [8].

It should be noted that cell death is a physiological process essential for the embryo development. Neuronal death was detected in the brain of the developing embryo during proliferation and cell migration [1]. In chicken embryos, ~45% cells in the retinal ganglion can die between E12 and E17 [5], whereas cell death in isthmo-optic nucleus between E12 and E17 is about 55% [2]. Death of nerve cells was observed during

G. Bunyatyan Institute of Biochemistry, National Academy of Sciences of Armenia; *Institute of Perinatology, Obstetrics and Gynecology, National Academy of Sciences of Armenia, Yerevan, Armenia. ***Address for correspondence:*** Kristine_danielyan@biochem.sci.am. K. E. Danielyan

0007-4888/11/1515-0579

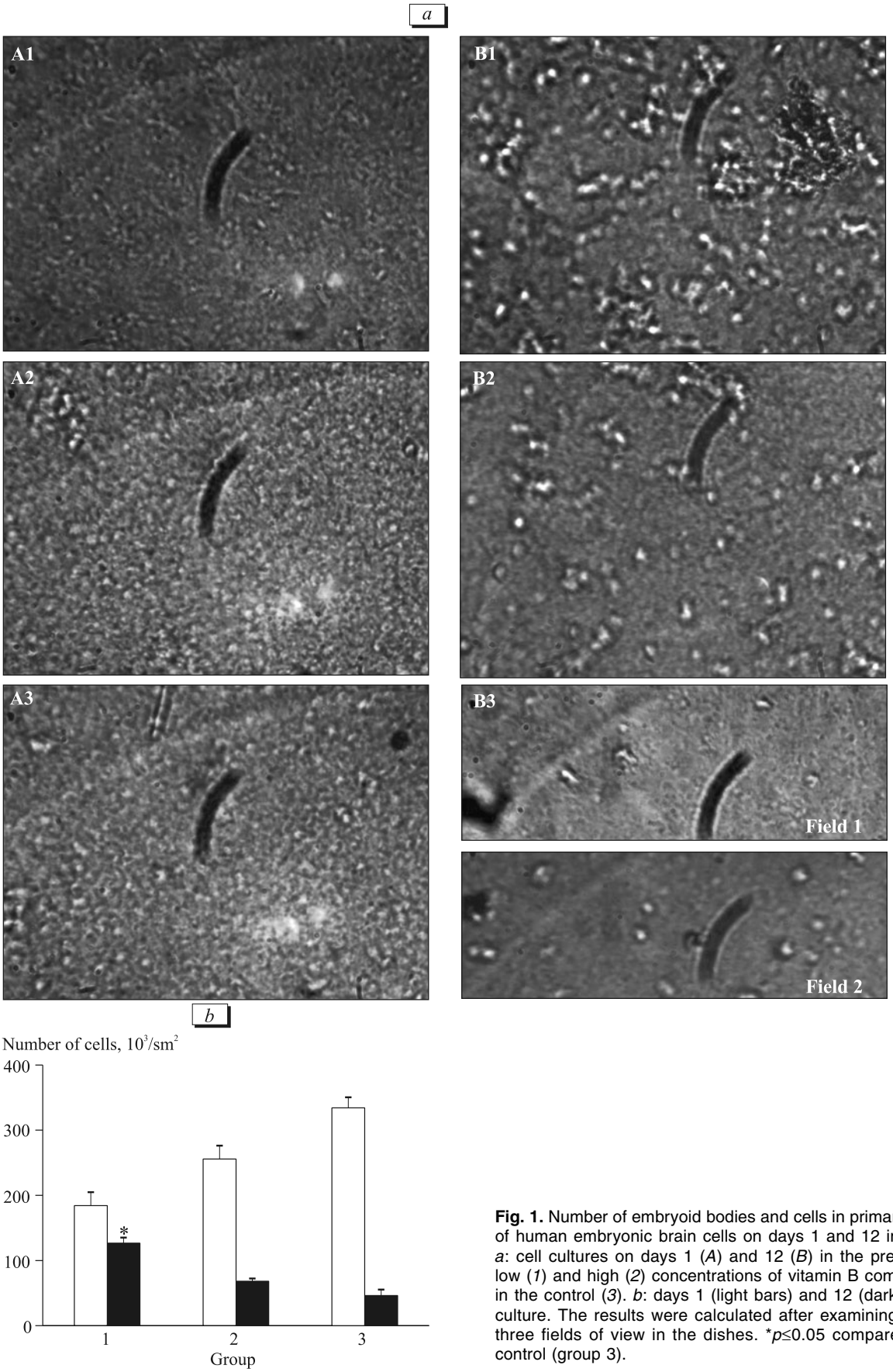

Fig. 1. Number of embryoid bodies and cells in primary culture of human embryonic brain cells on days 1 and 12 in culture. *a*: cell cultures on days 1 (*A*) and 12 (*B*) in the presence of low (*1*) and high (*2*) concentrations of vitamin B complex and in the control (*3*). *b*: days 1 (light bars) and 12 (dark bars) in culture. The results were calculated after examining at least three fields of view in the dishes. *$p \leq 0.05$ compared to the control (group 3).

the formation of intercellular connections with other neurons (synapses).

Here we studied the impact of two concentrations of the vitamin B complex on cell growth, development, and death of the human brain cells in culture.

MATERIALS AND METHODS

The cell suspension was prepared using HBSS (Hanks Balanced Salt Solution, Sigma). Then, 0.5 ml 0.1% trypan blue was transferred into a test tube, and 0.3 ml 10% BSA and 0.2 ml cell suspension were added. The mixture was incubated for 5 min, a few microliters suspension was transferred to a Goryaev chamber. Snapshots were obtained from areas with greatest cell density. Dead cells stained blue, while viable cells remained unstained [10].

We used the tissues obtained during induced abortion in humans (E90). Human embryonic brain was isolated and placed in a special medium (Neurobasal medium, NB, prenatal, Gibco Life Technologies) containing 0.05% BSA. After 20-min incubation (37°C) in NB containing 0.05% BSA and 0.15% trypsin, the brain tissue was transferred to a fresh medium, minced, and dissociated to suspension with Pasteur pipette (1, 5, and 10 ml). The supernatant was removed and the cell suspension was diluted with NB medium with 1% BSA. This procedure was repeated 3 times. Human brain cells were concentrated, washed, and seeded onto 35-mm Petri dishes at 37°C with 5% CO_2 precoated with poly-L-lysine (Sigma) dissolved in buffer containing 1% glucose, 0.001% gentamicin sulfate, 0.09% Na_2HPO_4, 0.4% KCl, 0.06% KH_2PO_4, 0.4% $MgSO_4 \times 7H_2O$. A day later, the medium was changed to NB-medium containing 2% B27-supplement (Gibco). The number of cells was counted after 16 h and on days 12 days after seeding [7].

A Bipolar PI microscope (PZO; magnification 60×1.25×40) was used. The photos were obtained after 16 hours and on the 12th day. The data were collected from 3 zones in each well.

In experimental group 1, vitamin B complex was added to the medium in a low concentration (0.003 mmol thiamine, 0.00066 mmol riboflavin, 0.0066 mmol pyridoxine, and 0.9 mmol nicotinamide per ml of medium); in group 2, vitamin B complex was added in a high concentration (0.46 mmol thiamine, 0.03 mmol riboflavin, 0.3 mmol pyridoxine, 3.9 mmol nicotinamide per ml of medium). In third (control) group, phosphate buffer was added.

For pairwise comparisons of the data, Student's *t* test was used. Comparisons between groups were performed using one-way ANOVA. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

In our experiments, we studied the dynamics of cell growth and death of the cells on days 1 and 12 after seeding. Two concentrations of vitamin B complex, high and low, were used (Fig. 1). Microscopy showed that the number of cells in three groups after 16 hours in groups 1, 2, and 3 (SEM) was 184.133±21.797, 255.666±20.637, and 334.181±16.430, respectively. On day 12, it was 126.74±8.691, 68.096±4.501, 46.25±9.535, respectively ($p<0.001$ compared to the control).

Both large and small fractions of cells were identified. Cells grown in the presence of the low concentration of vitamin B complex were significantly larger than in the control (Fig. 2). Large cell fraction

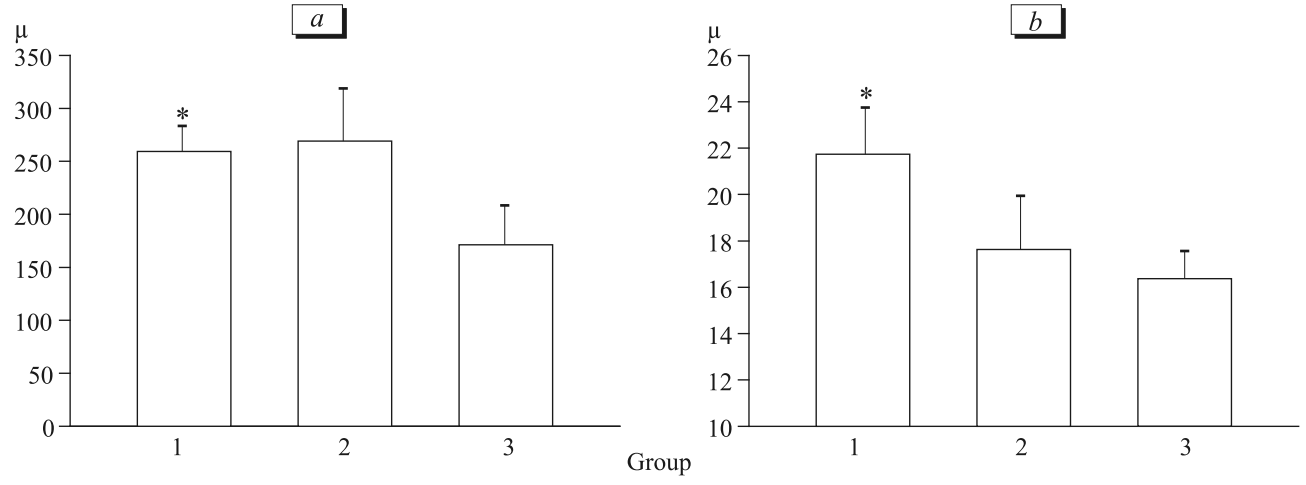

Fig. 2. Size of embryoid bodies and cells in primary culture of human embryonic brain cells on days 1 and 12 in culture. *a*: large cells, *b*: small cells. *$p<0.05$ compared to the control.

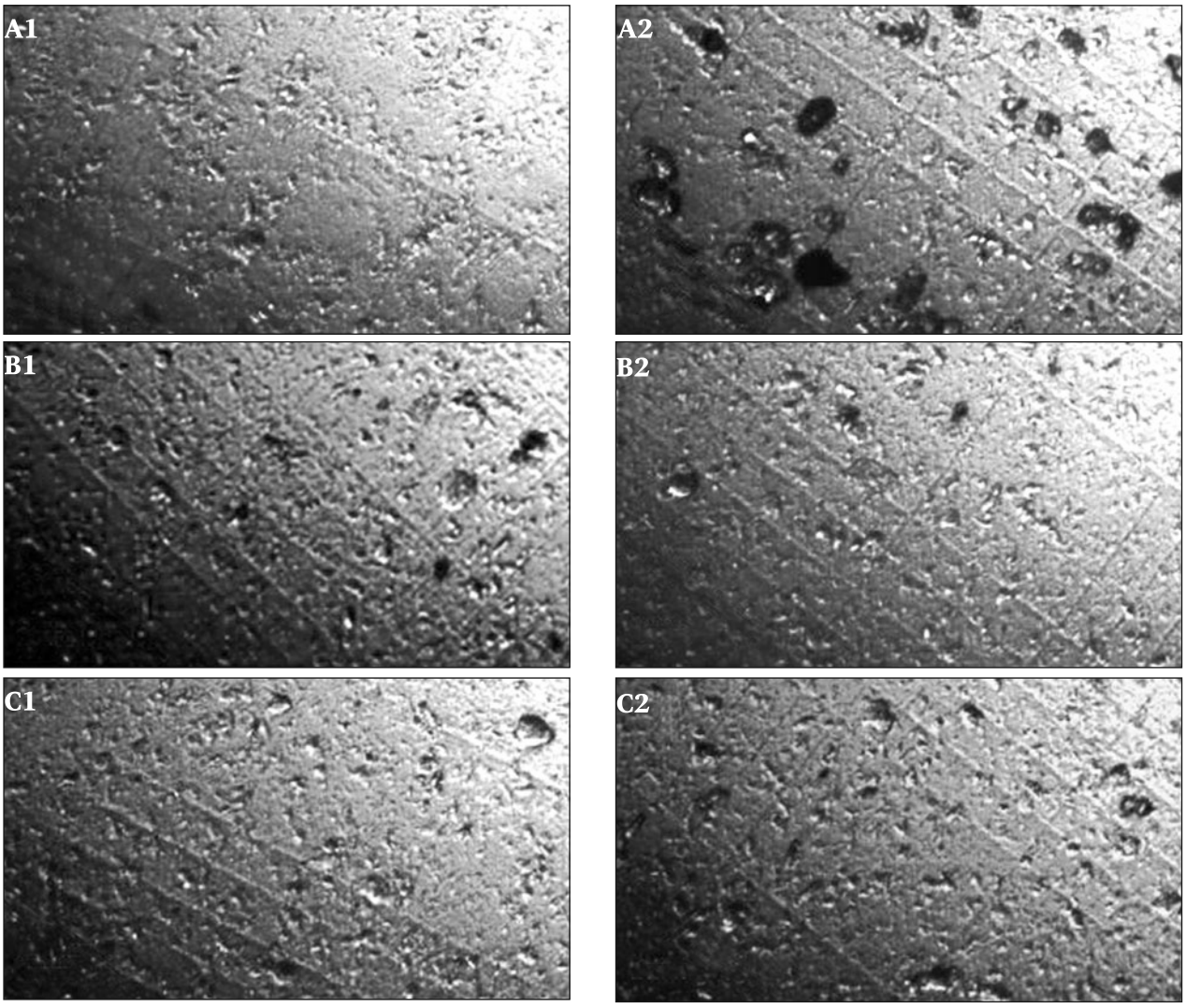

Fig. 3. Dead cells in primary culture of human fetal brain on day 12 in culture. The results were calculated after examiningat least three fields of view in the dishes. Cells were grown in medium with low (A1, A2) or high (B1, B2) concentration of vitamin B complex on day 12 in culture; C1 and C2: control.

in groups 1, 2, and 3 constituted 259.371±24.420, 268.992±49.450, and 171.099±36.620, respectively ($p \leq 0.02$ between groups 1 and 3). Small cells fraction was 21.738±1.961 (group 1), 17.629±2.302 (group 2), 16.3675±1.188 (group 3), $p \leq 0.05$ between groups 1 and 3. On day 12, large cells or cell groups stained with trypan blue, were detected in groups 1 and 2 (259.371±24.420 in group 1; 268.992±49.450 in group 2; 171.099±36.620 in group 3; $p \leq 0.05$ between groups 1 and 3; Fig. 3). In groups 1 and 2, large stained cells dominated over relatively small unstained cells, the difference was statistically significant ($p \leq 0.05$).

The percentage of dead cells within the groups was also calculated. It was found that the percentage of dead cells in experimental cultures treated with B-complex in different concentrations was much higher than in control (68% and 27.41% vc. 9.40%). cultures

Thus, vitamin B-complex in the low dosed promotes the development, maturation, and enlargement of brain cells from human embryo on the other, and increases the percent of dead cells on the other hand, which attests to accelerated maturation and metabolism of cells, or cell groups cell groups and clusters.

REFERENCES

1. A. J. Blaschke, K. Staley, and J. Chun, *Development*, **122**, No. 4, 1165-1174 (1996).
2. P. G. Clarke, L. A. Rogers, and W. M. Cowan, *Comp. Neurol*., **167**, No. 2, 125-142 (1976).
3. M. Y. Geng, H. Saito, and H. Katsuki, *Jpn. J. Pharmacol*., **68**, No. 3, 349-352 (1995).
4. M. Y. Geng, H. Saito, and H. Katsuki, *Neurosci. Res*., **24**, No. 1, 61-65 (1995).

5. W. F. Hughes and S. C. McLoon, *Exp. Neurol*., **66**, No. 3, 587-601 (1979).
6. C. A. Elvehjem, *Am. J. Public. Health Nations. Health*., **25**, No. 12, 1334-1339 (1935).
7. M. P. Mattson and B. Rychlik, *Brain Res*., **522**, No. 2, 204-214 (1990).
8. S. K. Mukherjee, L. K. Klaidman, R. Yasharel, and J. D. Adams, *Eur. J. Pharmacol*., **330**, No. 1, 27-34 (1997).
9. A. Patt, A. H. Harken, L. K. Burton, *et al*., *J. Clin. Invest*., **81**, No. 5, 1556-1562 (1988).
10. J. R. Tennant, *Transplantation*, **2**, 685-694 (1964).